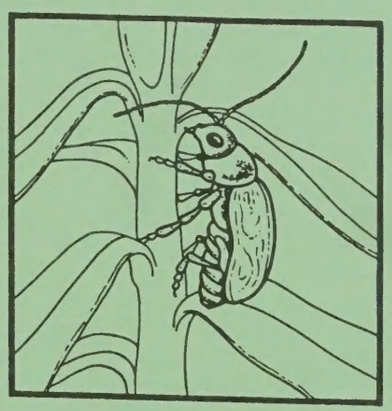
United States
Department of
Agriculture

**Animal and
Plant Health
Inspection
Service**

**Plant Protection
and Quarantine**

Biological Control of Leafy Spurge Project Manual



United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Plant Protection
and Quarantine

Biological Control
Operations

BIOLOGICAL CONTROL OF LEAFY SPURGE PROJECT MANUAL

Fiscal Year 1994

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INTRODUCTION

Orientation to the Biological Control Project Against Leafy Spurge (LS Project)

History

A native of Eurasia, leafy spurge (*Euphorbia esula*) was first discovered in North America at Newbury, MA in 1827. This Atlantic coast infestation was probably introduced from ship ballast. Infestations of leafy spurge in the Midwest and Western States, however, appear to be unrelated to the Atlantic coast infestation. Unlike the Atlantic coast infestation, which is suspected of originating in Western Europe, leafy spurge populations of the Great Plains are thought to have originated in Russia from importations of grains and grass seed.

Various cultural controls have been used in an attempt to eliminate or control leafy spurge. Deep cultivation is ineffective since the plant is able to recover after complete removal of the underground portion to a depth of 3 feet. Intensive cultivation may provide control in certain cropping systems by reducing available carbohydrates. Because of their ability to forage on leafy spurge, sheep and goats have also been suggested as a control measure. The exact extent of use of sheep and goats in an integrated control program remains unknown.

Chemical control of leafy spurge with various herbicides is one of the more widely used options. However, the use of herbicides over rough terrain and near water ways is limited, and the cost of herbicide applications to large areas becomes prohibitive. The integration of chemical control and range management practices with biological control strategies may prove to be a necessary and satisfactory method of controlling leafy spurge.

The following insects are currently being used (or have been used) as biological control agents of leafy spurge:

- Five *Aphthona* species (Coleoptera: Chrysomelidae) (flea beetles)
- *Oberea erythrocephala* (Coleoptera: Cerambycidae) (a root-boring beetle)
- *Spurgia esulae* (Diptera: Cecidomyiidae) (gall midge)

By itself, it is doubtful that any one of these insects could successfully control leafy spurge. However, a combination of biological control agents may achieve significant results. Additional insects and plant diseases are currently being evaluated and may be added to the arsenal of leafy spurge biological control agents. Before any biological control agent is imported, it is thoroughly evaluated to make sure it will feed only on leafy spurge (see Appendix 8).

The Great Plains Agricultural Council (GPC-14) Leafy Spurge Committee made a cooperative biological control program proposal in December 1985. This proposal outlined the need for an expanded establishment and redistribution effort to provide insects for large-scale release. Because of the proposal's recommendations, Plant Protection and Quarantine (PPQ) became involved in the Leafy Spurge program. PPQ's role has been to facilitate the entry of biological control agents into the United States and to establish insectary sites for the multiplication of insect biological control resources.

Economics

The introduction of leafy spurge into North America has caused serious problems for ranchers in Western and Midwestern States. Leafy spurge is an aggressive perennial weed which displaces other plants in pasture and rangeland habitats. Reductions of forage from 10 percent to 100 percent have been observed. Excluding sheep and goats, most range animals avoid leafy spurge. If eaten in sufficient quantities, leafy spurge can cause weakness or death of some animals.

Project Length

Project activity is scheduled for fiscal years (FY) 1994 and 1995.

FY 1994: In selected states in PPQ's Northeastern, Central, and Western Regions, the following will be accomplished:

- Selection of new insectary sites
- Evaluation of previous releases
- Release of new agents
- Redistribution of established biocontrol agents

FY 1995: The previous accomplishments for FY 1994 will be repeated in selected States in PPQ's Northeastern, Central, and Western Regions, but with decreasing emphasis on selection of new insectary sites, and increasing emphasis on redistribution of established biocontrol agents.

Who's Involved

The Agricultural Research Service (ARS), State departments of agriculture, universities, Extension Service and industry personnel, and other Animal and Plant Health Inspection Service (APHIS) staffs are contributing to the implementation of the LS Project.

The Project Leader is Paul Parker, who is located at the National Biological Control Laboratory, PPQ, APHIS, in Mission, Texas.

PPQ Entomologists are Robert Richard and Richard Hansen, who are located at the Bozeman Biological Control Facility (BBCF), in Bozeman, Montana.

Cooperators include PPQ line personnel, State departments of agriculture personnel, university researchers, and Extension Service personnel. State project coordinators are generally officers-in-charge at designated PPQ locations.

Goal and Objectives

The goal is to provide an effective, economical method for control of leafy spurge using biological control. The project objectives cover the following topics:

- Distribution of leafy spurge
- Establishment of field insectary sites
- Collection of biological control agents
- Release of biological control agents
- Recovery of biological control agents
- Importation of biological control agents
- Collection of soil samples at release sites
- Redistribution of biological control agents
- Development of predictive models
- Education and public awareness of leafy spurge
- Leafy spurge range management study
- Laboratory mass rearing of biological control agents
- Evaluation of leafy spurge biological control
- Cooperator involvement and personnel training

INTRODUCTION
Roles and Responsibilities

Cooperators

PPQ line personnel, State Departments of Agriculture personnel, university researchers, and Extension Service personnel will provide the technical assistance in the field to do the following tasks:

1. Select new insectary sites.
2. Evaluate previous releases.
3. Release new agents.
4. Redistribute established biocontrol agents.

State Project Coordinators

State project coordinators are generally officers-in-charge at designated PPQ locations. They are responsible for the following tasks:

1. Assign cooperators designated work areas.
2. Plan and coordinate work hours needed for cooperators to accomplish the tasks listed above.
3. Evaluate information gathered by cooperators to ensure accuracy and completeness.
4. Communicate with the project leader about the project's progress.

Project Leader

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National Biological Control Laboratory
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Commercial: (210) 580-7301
FAX: (210) 580-7300
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The Project Leader's responsibility is to coordinate all efforts while meeting the objectives of the project.

Bozeman Biological Control Facility (BBCF)

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USDA, APHIS, PPQ
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Montana State University
Bozeman, MT 59717-0278
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E-Mail: !a348bcbozema

If you have any questions about the Biological Control Project Against Leafy Spurge, make BBCF your first contact.

INTRODUCTION
How to Use This Manual

Use the LS Project Manual as an on-the-job reference when selecting new insectary sites, evaluating previous releases, releasing new agents, and redistributing established biocontrol agents.

Each tabbed section is independent, containing step-by-step procedures.

Each section has an Introduction which contains general information relating to the section's main content.

The Overview is a list of steps described in the section. If you are familiar with the steps, you can use the Overview as a checklist.

Use the Appendixes as they relate to the other sections of the manual. In some places an Appendix is referenced; in other places it is assumed that you accessed an Appendix to get the necessary information.

If the Contents section is not specific enough, use the Index to find a topic and its page number.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)
Introduction

When to Establish

The Bozeman Biological Control Facility (BBCF) will contact you to let you know when the time is right for establishing a field insectary in your State.

Purpose

The purpose of a field insectary is to develop a field location where biological control agents can be collected in large numbers. The desired goal is to redistribute these agents to other infestations of leafy spurge, where the agents have not been released in the past or where high populations of the agents have not developed. For a description of the APHIS process to release exotic natural enemies of weeds for establishment and redistribution, see Appendix 5.

Overview

If you are familiar with the process, you can use the following overview as a checklist for establishing a field insectary.

1. Select a suitable site for the insectary.
2. Fill out a Field Insectary Site Preliminary Information Sheet (FISPIS).
3. Release the biocontrol agents at the Phase 1 FIS.
4. Fill out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).
5. Collect samples of biocontrol agents from the Phase 1 FIS.
6. Fill out a Leafy Spurge Biocontrol Agent Recovery and Sampling Report.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)

Select a Suitable Site for the Insectary

Introduction

You must plan very carefully when you select a site for establishing a field insectary. You must be able to identify leafy spurge, and determine if the site is suitable by considering all the criteria listed in Step 2 below. For help in identifying leafy spurge plants, see Appendix 1. BBCF will contact you to let you know when the time is right for establishing a field insectary in your State.

Step 1

Contact State department of agriculture personnel and county extension educators to determine where large stands of leafy spurge are located.

Step 2

Visit prospective sites to evaluate their suitability for establishing a field insectary, using the following criteria:

- Stand density:
  --Moderately dense stands of leafy spurge are best. Ideally, individual stems should be 1-2 inches apart; never more than 6 inches apart. The best plant height is 15-24 inches. For a graphical illustration of ideal stem spacing and height, see Appendix 6.

- Sunlight:
  --Open fields without shade are best (the biocontrol agents like sun).
  --The site should be at least 100 yards from any trees.

- Exposure:
  --Southern exposures are best. Neutral exposures (flat locations) are acceptable, while northern exposures are least desirable.

- Soil type:
  --Moderately textured loams are best. Clay soils are not as good.
  --The soil should be moderately to well drained, and the area should be free from seasonal flooding.

- Grazing:
  --The area should be free from grazing.

- Field size:
  --The field should be at least 2 acres large, preferably 5 acres or larger.

- Pesticide use:
  --The site should not be exposed to insecticides.
  --No herbicides should be used within 500 ft. of the release point.

- Land ownership:
  --Public land is usually a better choice than private land, because of longevity of management.

Step 3

After you have visited several prospective sites, choose a site that has most of the desirable characteristics listed above.

Step 4

Contact the landowner or land manager in person or by telephone. Identify yourself (give a business card if available). Give the landowner a copy of Program Aid Number 1435, Biological Control of Leafy Spurge, to help explain the importance of the LS Project.

Step 5

Ask permission to establish a field insectary site. Make sure that the landowner or land manager is aware of, and willing to make the 5-year commitment described in item 25 on the Field Insectary Site Preliminary Information Sheet.

Step 6

Go to the next section--Fill out a Field Insectary Site Preliminary Information Sheet.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)

Fill Out a Field Insectary Site Preliminary Information Sheet (FISPIS)

Introduction

The FISPIS was designed to serve the following two basic functions (see an example in Appendix 7):

1. The FISPIS can aid in the "pre-season" process of selecting FIS release locations. For example, if 10 locations are being considered for 5 insect releases, the information contained in the FISPIS can help select the best locations for the releases.

2. The FISPIS provides a variety of useful biological and physical information about the release location that, when later coupled with FIS insect population data, can determine what site characteristics are best suited for biocontrol agents. This information, combined with the soil sample data collected in 1992, can help guide the placement of future insect releases. If you have not already done so, **complete this form at the time you release biocontrol agents at a Phase 1 FIS.**

Step 1

Find the green form titled USDA-APHIS BIOCONTROL OF WEEDS: Field Insectary Site Preliminary Information Sheet (FISPIS). It should be included with the material you received from BBCF.

Step 2

Record the following information on the FISPIS:

- Target Weed: Place an "X" in the appropriate space for leafy spurge.
- Release code: Leave blank; this will be assigned by BBCF.
- Contact person: Record your name, address, and phone number.
- Legal landowner: Record the landowner's name, address, and phone number.
- Site Location: Select a site name, and identify the state, county, township, range, section, and quarter-section of your site. Determine and record the latitude and longitude of your site using the Transpak II Global Positioning System (GPS) unit (see Appendix 4). The manufacturer has provided detailed instructions for operating the unit. Please review these instructions before you take any readings.

Step 3

Draw a map on the back of the FISPIS, or attach a map that shows road access to the site.

Step 4

Record on the FISPIS the requested data pertaining to physical, biological, cultural, and other site characteristics. If you are not sure how to answer a question, leave it blank.

Step 5

Distribute the FISPIS as follows:

If you are:	And you are located at:	Then:
An officer-in-charge (OIC)	→	1. RETAIN a photocopy of the FISPIS. 2. MAIL the original to BBCF within 1 week of your release along with Forms AD-943 and AD-943A.
A PPQ officer	The same duty station as your OIC	
	A duty station physically removed from your OIC's duty station	1. RETAIN a photocopy of the FISPIS. 2. MAIL a photocopy to the OIC covering your State. 3. MAIL the original to Bozeman within 1 week of your release along with Forms AD-943 and AD-943A.
A State cooperator	→	

Step 6

Go to the next section--Release the Biocontrol Agents at the Phase 1 FIS.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)
Release the Biocontrol Agents at the Phase 1 FIS

Introduction

BBCF will work with you in choosing when and where to receive your shipment of biocontrol agents. If there is a suitable delivery point closer to the release site than your office, BBCF may opt to ship the biocontrol agents to that location.
See Appendix 2 for photographs and narrative descriptions of the biocontrol agents. You can find information on the insects' life cycles and how they damage leafy spurge plants in Appendix 2. See Appendix 3 for a pronunciation guide.

Releasing *Aphthona* spp. (Flea Beetles)

Step 1:

Upon receipt from BBCF, open the shipping package and place the canister containing the biocontrol agents in a refrigerator **(NOT FREEZER!)** at 40°-50°F until you are ready for transport to the field. **YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!**

Step 2:

Place the canister in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the canister on top of the foam beads or newspaper. **DO NOT ALLOW THE CANISTER TO DIRECTLY CONTACT THE BLUE ICE PACK!**

Step 3:

Drive to the release site. **DO NOT** wait for good weather!

Step 4:

Mark the release point by driving a metal stake securely into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the beetles are alive, sacrifice two or three for your voucher sample.

Step 6:

Place the beetles you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Write the name of the biocontrol agent on a small paper label and place it **inside** the vial. By doing this, you will have identified specimens to use as a reference when you collect beetles for the recovery report in successive years. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on leafy spurge plants within a 3-ft. radius of the stake.

Step 9:

Return the empty canister to your vehicle (do not leave the canister at the release site).

Step 10:

Go to the next section--Fill out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

Releasing *Spurgia esulae* (Bud Gall Midge)

Step 1:

Upon receipt from BBCF, open the shipping package and place the canister containing the biocontrol agents in a refrigerator **(NOT FREEZER!)** at 40°-50°F until you are ready for transport to the field. **YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!**

Step 2:

Place the canister in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the canister on top of the foam beads or newspaper. **DO NOT ALLOW THE CANISTER TO DIRECTLY CONTACT THE BLUE ICE PACK!**

Step 3:

Drive to the release site. **DO NOT** wait for good weather!

Step 4:

Select a release point that has an overstory of taller spurge stems to provide shade. This will help to delay drying out of the galls.

Step 5:

Mark the release point by driving a metal stake securely into the ground.

Step 6:

Dig a hole about 4-5 inches deep using a trowel or small shovel.

Step 7:

Fill the hole up with water.

Step 8:

Place a bundle of 20 terminal galls in the hole, so that about two-thirds of the stem length is underground. Firm up the soil around the bundles. You may place several bundles in close proximity.

Step 9:

Retain two or three galls for your voucher sample. Place the galls in a small screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 10:

Write the name of the biocontrol agent on a small paper label and place it **inside** the vial. By doing this, you will have identified specimens to use as a reference when you collect galls for the recovery report in successive years. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 11:

Return the empty canister to your vehicle (do not leave the canister at the release site).

Step 12:

Go to the next section--Fill out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)

Fill out Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data)

Introduction

These forms are used for recording the details of biocontrol agent shipments and releases. The information will be published by the Beneficial Insect Introduction Laboratory of the Agricultural Research Service, and you will be credited with the release. See an example of each form in Appendix 7.

Filling Out Forms

AD-943:

Blocks 1 through 18 will be completed for you.
Fill out **Blocks 19 through 33** with the details of your release.
Block 19: Enter the date you received the shipment.
Block 20: Record the number and stages of biocontrol agents you received. Use the codes on the reverse of the form.
Block 21: Note the condition of the material upon its arrival. Examples might be, "Insects looked lively," "90 percent of insects dead," or "Insects alive but inactive."
Block 22: Record the number of specimens you retained for your voucher samples.
Block 23: Check box A (Immediate release).
Block 24: Enter "N/A" (not applicable).
Block 25: Check the "Field" box for each site. Use Section B of Form AD-943A if you have more than three release sites. **Do not use Section C.**
Block 26: Record the location of each site. Please provide township, range, and section information down to quarter section. For example, Sec. 32 SE is the southeast quarter of section 32. Record the latitude-longitude data as determined by the Transpak II GPS unit. Complete Section A, Form AD-943A. **NOTE:** If you have already provided this information on the FISPIS, it will NOT be necessary for you to do so again. Include a photocopy of a map of each release site or draw a map that relates the release site to some topographical feature.
Block 27: Record the number and stages of biocontrol agents you released. Use the codes on the reverse of the form.
Block 28: Enter the date you released the biocontrol agents.
Block 29: Enter *Euphorbia esula* as the primary target host (line A.).
Block 30: Enter "N/A."
Block 31: Enter the name and affiliation of the actual releaser.
Block 32: Describe any special conditions at the time of the actual field release. This might include weather conditions (e.g., "Released in heavy rain, 45°F"), steps taken when you release the agents (e.g., "Release marked with blue-painted stake"), or cooperating personnel involved in, or present at, the release, especially if all their names do not fit in **Block 31**.
Block 33: Enter your name and date.

AD-943A:

Shipper's File Number: Copy this number from **Block 3** of Form AD-943.
Section A--
Township, route no., Farmer's name, etc. Map of release site.: Draw a map of the release site in the space provided.
WEATHER (including **TEMP., WIND, SKY**): Fill in these blocks using the example in Appendix 7 as a guide.
TIME OF RELEASE: Fill in this block using the example in Appendix 7 as a guide.
CONDITION OF CROP FIELD: Fill in this block using the example in Appendix 7 as a guide.
CONDITION OF RELEASE MATERIAL: Fill in this block using the example in Appendix 7 as a guide.

Mailing Forms

Return Forms AD-943 and AD-943A within 1 week of your release to BBCF (envelope enclosed). **PLEASE REMEMBER TO APPLY POSTAGE!**

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)
Collect Samples of Biocontrol Agents From the Phase 1 FIS

Introduction

Sampling is a tool that permits us to estimate the size of an insect population, and to describe some of the characteristics of that population. Reliable estimates of agent population size are essential in developing a sound redistribution strategy.

When to collect

BBCF has developed a three-time sampling schedule based on an emergence model and temperature data from each State. You will need to collect samples 2 weeks before anticipated "peak" emergence, at "peak" emergence, and two weeks after "peak" emergence. BBCF will let you know when to collect samples.

Collecting *Aphthona* spp. (Flea Beetles)

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Sunny, warm (>65°F) day; calm or with just a slight breeze.
- Dry vegetation. Plan to arrive at the release site **NO EARLIER THAN 10 a.m.** (heavy dew may interfere with collection earlier in the morning).

Step 2:

Prepare to collect the flea beetles. Check to make sure you have the following items:

- 15-inch diameter sweep net
- 70 percent ethyl or isopropyl alcohol
- Small glass screw-cap vial
- Pencil(s)

Step 3:

Drive to the Phase I FIS (your previous release site). Use as a guide the map on the back of your photocopy of the Field Insectary Site Preliminary Information Sheet (FISPIS).

Step 4:

Locate the metal stake that marks the exact release point.

Step 5:

Spend a minute or two looking over the site to note if you can readily see adult *Aphthona* spp. beetles on leafy spurge plants.

Step 6:

Using the sweep net, collect samples at five points along four lines in N, S, E, and W directions from the original release point.

1. For each line, begin as close to the release point as possible.

2. Make **four** sweeps in front of you (back and forth twice). Sweep the net vigorously through the vegetation in a downward arc, as close to the ground as possible.

3. Carefully examine the net and count the *Aphthona* spp. beetles present.

4. Empty the net to release the beetles you have counted.

5. Record the number of beetles you counted in the appropriate block on the back of the Leafy Spurge Biocontrol Agent RECOVERY AND SAMPLING REPORT - 1993 (see Appendix 7).

6. Move two paces (5 to 6 feet) out and repeat the procedure.

7. Continue until five points have been sampled, then repeat over the remaining cardinal directions.

Step 7:

During the sweeping process, collect a **maximum** of 15 beetles from the site and place them in 70 percent ethyl or isopropyl alcohol in a **glass** screw-cap vial.

Step 8:

Label the vial with the **release code, site name, and date.** Place the label **inside** the vial. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 9:

Mail your samples to BBCF **with the Leafy Spurge Biocontrol Agent Recovery and Sampling Report.**

Step 10:

If you do not collect any samples, return only the completed Leafy Spurge Biocontrol Agent RECOVERY AND SAMPLING REPORT.

Collecting *Spurgia esulae* (Bud Gall Midge)

Step 1:

Prepare to collect the galls. Check to make sure you have the following items:

- 70 percent ethyl or isopropyl alcohol
- Small glass screw-cap vial
- Pencil(s)

Step 2:

Drive to the Phase I FIS (your previous release site). Use as a guide the map on the back of your photocopy of the FISPIS.

Step 3:

Locate the metal stake that marks the exact release point.

Step 4:

Visually survey an area roughly 50-75 feet in diameter and centered at the original release point. Estimate the number of galls present in the 50-75 ft. diameter area and check the appropriate blank on the Leafy Spurge Biological Agent Recovery and Sampling Report (None, <25 galls, 25-100 galls, or >100 galls).

Step 5:

Collect a **maximum** of five galls from the site and place them in 70 percent ethyl or isopropyl alcohol in a glass screw-cap vial.

Step 6:

Label the vial with the **release code, site name, and date.** Place the label **inside** the vial. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 7:

Mail your samples to BBCF **with the Leafy Spurge Biocontrol Agent Recovery and Sampling Report.**

Step 8:

If you do not collect any galls, return only the completed Leafy Spurge Biocontrol Agent RECOVERY AND SAMPLING REPORT.

ESTABLISHING A PHASE 1 FIELD INSECTARY SITE (FIS)
Fill Out a Leafy Spurge Biocontrol Agent Recovery and Sampling Report

Introduction This form documents the results of your sampling efforts. It is very important to record your observations on this form because BBCF will use the data to estimate the population size of biocontrol agents. See Appendix 7 for an example.

Step 1 Record the information pertaining to location of the release site:

Release code: Enter the number provided to you by BBCF. BBCF assigns this code after receiving your FISPIS and Forms AD-943 (Biological Shipment Record--Non-Quarantine) and AD-943A (Supplemental Data).

State and County: Record the state and county in which the release site is located.

Site name: Copy the site name from the appropriate FISPIS or AD-943.

USGS coordinates (United States Geological Survey): Provide township, range, and section information down to quarter section.

Latitude and Longitude: Record the latitude-longitude data as determined by the Transpak II GPS unit. If you did not record these data at the time of initial release, do so now.

Step 2 Indicate which insect was released, the date of the original release, and whether the release was in a cage or in the open.

Step 3 Record the requested **SAMPLING INFORMATION:**

SAMPLING DATE and SAMPLING TIME (approx.): Enter the date (month, day, year), and the approximate local time you began sampling.

Weather conditions, Air temperature (F°), and Wind: Give your best estimations of these conditions. Ideally, measure the temperature with a thermometer.

Visual observation of insect, before sweeping: Just check for obvious presence of the insect. Do not spend more than 5 minutes looking.

Number of Net sweeps: If you are sampling for *Aphthona* spp., enter 80 (you should have made 4 sweeps at each of 5 sampling points along 4 lines).

For *Spurgia esulae*, number of galls observed: If you are sampling for *Spurgia esulae*, record the number of galls you observed within the 50-75 ft. diameter survey area.

Observer, Affiliation, and Phone: Enter your name, organization, and phone number.

Step 4 See the reverse side of this form for the diagram of the sampling procedure and chart for recording insect counts. On the diagram sketch any leafy spurge death or suppression that you observe. Record your insect counts in the appropriate blocks on the chart.

ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS)
Introduction

When to Establish

The Bozeman Biological Control Facility (BBCF) will let you know when the time is right for establishing a Phase 2 Field Insectary in your state.

Purpose

Phase 2 Field Insectary Sites will serve two purposes:

1. To function as the source of additional natural enemies in 3 to 5 years for continued redistribution in Phase 3.

2. To serve as demonstration plots, showing the potential impact of the natural enemy on leafy spurge.

Overview

If you are familiar with the process, you can use the following overview as a checklist for establishing a Phase 2 Field Insectary.

1. Select a suitable site for the insectary.

2. Collect biocontrol agents from a Phase 1 FIS for redistribution to the Phase 2 FIS.

3. Release the biocontrol agents at the Phase 2 FIS.

ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS)

Select a Suitable Site for the Insectary

Introduction

Establishing a Phase 2 FIS will be a cooperative effort by APHIS and State departments of agriculture and/or research cooperators. You must plan very carefully when you select a Phase 2 FIS, just as you did when you selected the Phase 1 FIS. BBCF will contact you to let you know when the time is right for establishing a Phase 2 FIS in your State.

Step 1

Contact State department of agriculture personnel and county extension educators. Tell them you have been informed by BBCF that your Phase 1 FIS has been successfully established, with enough biocontrol agents for redistribution.

Step 2

With input from State personnel who are knowledgeable about leafy spurge infestation, visit prospective sites to evaluate their suitability for establishing a Phase 2 FIS, using the following criteria:

- Stand density:
  --Moderately dense stands of leafy spurge are best. Ideally, individual stems should be 1-2 inches apart; never more than 6 inches apart. The best plant height is 15-24 inches. For a graphical illustration of ideal stem spacing and height, see Appendix 6.

- Sunlight:
  --Open fields without shade are best (the biocontrol agents like sun).
  --The site should be at least 100 yards from any trees.

- Exposure:
  --Southern exposures are best. Neutral exposures (flat locations) are acceptable, while northern exposures are least desirable.

- Soil type:
  --Moderately textured loams are best. Clay soils are not as good.
  --The soil should be moderately to well drained, and the area should be free from seasonal flooding.

- Grazing:
  --The area should be free from grazing.

- Field size:
  --The field should be at least 2 acres large, preferably 5 acres. Fields larger than 5 acres are also acceptable.

- Pesticide use:
  --The site should not be exposed to insecticides.
  --No herbicides should be used within 500 ft. of the release point.

- Land ownership:
  --Public land is usually a better choice than private land, because of longevity of management.

Step 3

After you have visited several prospective sites, choose a site that has most of the desirable characteristics listed above.

Step 4

Contact the landowner or land manager in person or by telephone. Identify yourself (give a business card if available). Give the landowner a copy of Program Aid Number 1435, Biological Control of Leafy Spurge, to help explain the importance of the LS Project.

Step 5

Ask permission to establish a field insectary site. Make sure that the landowner or land manager is aware of, and willing to make the 5-year commitment described in item 25 on the Field Insectary Site Preliminary Information Sheet.

ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS)

Collecting Biocontrol Agents From a Phase 1 FIS for Redistribution to a Phase 2 FIS

Introduction

You must have a successfully established Phase 1 FIS in order to collect biocontrol agents for redistribution. Based on the data you provided on the Leafy Spurge Biocontrol Agent RECOVERY AND SAMPLING REPORT -1993, BBCF will confirm that you have a large enough population from which to collect.

Collecting *Aphthona* spp. (Flea Beetles)

Step 1:

Wait for a good day to collect the biocontrol agents. The following conditions are ideal:

- Sunny, warm (>65°F) day; calm or with just a slight breeze.
- Dry vegetation. Plan to arrive at the release site **NO EARLIER THAN 10 a.m.** (heavy dew may interfere with collection earlier in the morning).

Step 2:

Prepare to collect the flea beetles. Check to make sure you have the following items:

- 15-inch diameter sweep net
- Graduated vial
- Shipping cartons (paper ice cream type)
- Cooler and the blue ice pack
- Clippers for collecting leafy spurge shoot tips
- Cardboard box for mailing flea beetles
- Masking tape
- Pencil(s)

Step 3:

Drive to the Phase 1 FIS (your previous release site). Use as a guide the map on the back of the Field Insectary Site Preliminary Information Sheet.

Step 4:

Using the net, sweep vigorously through the vegetation to collect as many beetles as possible.

Step 5:

Use your clippers to collect leafy spurge shoot tips that **DO NOT HAVE FLOWERS OR SEED CAPSULES.** Do **NOT** include any flowers, seeds, or roots with the shoot tips. Collect enough shoot tips to make the shipping carton half full. Place the shoot tips in the carton.

Step 6:

Scoop beetles out of the net with the graduated vial. Fill the vial up to the desired mark with beetles. The vial, marked in 500-beetle increments, is illustrated in Appendix 2.

Step 7:

Empty the vial into the shipping carton with the shoot tips. **DO NOT PUT MORE THAN 500 BEETLES IN A PINT CARTON.** Put the top on securely.

Step 8:

Repeat this process for additional cartons if you are collecting for more than one Phase 2 FIS.

Step 9:

Seal the carton(s) with masking tape. Do **NOT** punch holes in the carton.

Step 10:

Label the carton(s)--write on the lid the species, the number of beetles you collected, and the date.

Step 11:

Place the shipping carton in the cooler with the blue ice pack. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the shipping carton on top of the foam beads or newspaper. **DO NOT ALLOW THE SHIPPING CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!**

Step 12:

Give the cooler containing the shipping carton to the state cooperator who will be working with the Phase 2 FIS, or mail the cooler in the cardboard box to the cooperator, if necessary. **THE COOPERATOR MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!**

Collecting *Spurgia esulae* (Bud Gall Midge)

This activity is not scheduled for FY 1994. The BBCF is developing a procedure for collecting and redistributing *Spurgia esulae* galls.

ESTABLISHING A PHASE 2 FIELD INSECTARY SITE (FIS)

Release the Biocontrol Agents at a Phase 2 FIS

Introduction

RELEASING BIOCONTROL AGENTS AT A PHASE 2 FIS IS A COOPERATOR ACTIVITY. The steps for releasing biocontrol agents at a Phase 2 FIS are the same as the steps for releasing biocontrol agents at a Phase 1 FIS. You will not, however, release *Spurgia esulae* at a Phase 2 FIS in FY 1994.

Releasing *Aphthona* spp. (Flea Beetles)

Step 1:

After you receive the *Aphthona* spp. from the collector, open the shipping package and place the carton containing the biocontrol agents in a refrigerator **(NOT FREEZER!)** at 40°-50°F until you are ready for transport to the field. **YOU MUST RELEASE THE BIOCONTROL AGENTS WITHIN 24 HOURS OF RECEIPT!**

Step 2:

Place the carton in a cooler with the blue ice pack for transport to the field. Position the blue ice pack in the bottom of the cooler, and put styrofoam beads or crumpled newspaper on top of the blue ice. Place the carton on top of the foam beads or newspaper. **DO NOT ALLOW THE CARTON TO DIRECTLY CONTACT THE BLUE ICE PACK!**

Step 3:

Drive to the release site. **DO NOT** wait for good weather!

Step 4:

Mark the release point by driving a metal stake securely into the ground.

Step 5:

Count and retain dead biocontrol agents for voucher samples. If all of the beetles are alive, sacrifice 2 or 3 for your voucher sample.

Step 6:

Place the beetles you retained in a small glass screw-cap vial containing 70 percent ethyl or isopropyl alcohol.

Step 7:

Label the vial so you will have these specimens as a reference. **CAUTION:** Always use a pencil to fill out labels. Ink from pens and markers bleeds and dissolves in alcohol.

Step 8:

Release the biocontrol agents on leafy spurge plants within a 3-ft. radius of the stake.

Step 9:

Return the empty canister to your vehicle (do not leave the canister at the release site).

Releasing *Spurgia esulae* (Bud Gall Midge)

This activity is not scheduled for FY 1994. The BBCF is developing a procedure for collecting and redistributing *Spurgia esulae* galls.

APPENDIX 1: LEAFY SPURGE PLANTS

Introduction

Use this appendix to help identify leafy spurge plants and infested rangeland. The last three photographs illustrate the effectiveness of *Aphthona nigriscutis* in controlling leafy spurge. The number in [] corresponds to the photograph number. The photographs are located in the back of this appendix.

***Euphorbia esula* (Leafy Spurge) [1-1], [1-2]**

Leafy spurge is a deep-rooted perennial member of the Euphorbiaceae family. The yellowish-green inflorescence, termed a cyathium, and the milky latex sap are characteristic for many members of the spurge family.

Leafy Spurge Infested Range [1-3]

Leafy spurge is an aggressive perennial weed which tends to displace other vegetation in pasture and rangeland habitats.

Leafy Spurge Pre-Release Density [1-4]

This photograph shows a typical leafy spurge density before release of biocontrol agents.

Leafy Spurge Suppression [1-5]

Two years after the initial release of *Aphthona nigriscutis*, the suppression of leafy spurge is evident. Note the rock ledge in center, for comparison with photograph **[1-6]**. Control (>90 percent reduction in leafy spurge stem density) was observed over an area of abut 240 m^2, centered roughly at the release point.

Leafy Spurge Suppression and Death [1-6]

Four years after the initial release of *Aphthona nigriscutis*, further suppression and death of leafy spurge is obvious. Compare the area of dead leafy spurge plants around the rock ledge to the same area in photograph **[1-5]**. Control was observed over an area of about 5,300 m^2 (1.3 acres).

1-1

1-2

1-3

1-4

1-5

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APPENDIX 2: BIOLOGY AND IDENTIFICATION OF BIOCONTROL AGENTS

Introduction

Use this appendix to help identify the biocontrol agents of leafy spurge. The appendix includes photographs as well as narrative descriptions of these biocontrol agents. The number in brackets [ ] corresponds to the photograph number. The photographs are located in the back of this appendix.

***Aphthona* spp. (Flea Beetles)**

Life Cycle:

All species currently being distributed in the United States have one generation per year. Like all beetles, *Aphthona* species exhibit complete metamorphosis and four life stages (egg, larva, pupa, and adult). Adult beetles are present for a month to several months during the summer, depending on location. Adults feed on leafy spurge leaves and flowers, causing irregular holes and blotches that often give the leaves a brown, "shredded" appearance. At high population densities, adult beetles may completely defoliate leafy spurge stems.

Mating and egg laying occur throughout the adult activity period. *Aphthona* females lay their eggs in the soil near the base of a leafy spurge stem, or on spurge stems near the soil surface. Individual fertility is highly variable. Generally, an adult female will lay 50-200 eggs. Larvae hatch in about 2 weeks and burrow down into the soil. *Aphthona* larvae feed on leafy spurge roots, at first consuming only the smallest roots. Then, as the larvae develop, they feed on progressively larger roots. Typically, larvae burrow in the soil around leafy spurge roots, but mature larvae may burrow inside and hollow out large spurge roots and root buds. *Aphthona* larvae enter a period of dormancy in the fall, overwinter, and resume feeding and development the following spring.

When the larvae complete feeding, they construct a soil "cell" and molt to the pupal stage. Pupal development lasts several weeks to a month or more, depending on temperature. Newly-hatched adults then emerge from the soil and begin feeding on leafy spurge foliage.

Identification:

Adults: Adults are the only *Aphthona* life stage that occurs outside the soil. Thus, they are the easiest life stage to collect, and serve as the target of sampling programs to assess *Aphthona* populations. A graduated vial [2-13] for "measuring" flea beetles (provided by Bozeman Biological Control Facility (BBCF)) speeds up the collection process. To use the vial, refer to the section, Collecting Biocontrol Agents From a Phase 1 FIS for Redistribution to a Phase 2 FIS.

Beetles are most active and, therefore, most visible on warm, sunny days. They are much less active and less visible on cloudy or rainy days, when they often week protected locations on spurge plants or in ground litter. The "shredded" appearance of leafy spurge foliage fed upon by *Aphthona* adults may indicate their presence, but this damage is conspicuous only at relatively high population densities.

Separation of adults of *Aphthona* flea beetles is difficult because they are small and often similarly colored. Adults of all *Aphthona* species are small beetles, typically 2-4 mm (about one-eighth in.) long, that "hop" but rarely fly if disturbed. At a given location, the smallest flea beetles present are usually males.

1. Adults of *Aphthona cyparissiae* are a yellowish-brown or bronze color [2-1]. This coloration is very similar to that of *A. nigriscutis*, but the dark dorsal "spot" that is clearly visible on *A. nigriscutis* is much fainter or absent altogether on *A. cyparissiae*. The feeding pattern of adult *A. cyparissiae* on leafy spurge plants [2-2] is typical of that exhibited by all *Aphthona* adults, but is often noticeable only with larger populations.

2. *Aphthona czwalinae* is a uniform shiny, metallic black color [2-3]. Adults of *A. lacertosa* are a similar shiny black color, and the coloration of the hind leg may differ slightly from that of *A. czwalinae*. However, this characteristic is not reliable, so these two species are also very difficult to separate without taxonomic expertise.

3. *Aphthona flava* beetles are a brighter orange or orange-brown color [2-4], both on the upper and lower surfaces and the legs. *A. flava* is also the largest of the five *Aphthona* beetles, as adults typically approach 4 mm in length.

4. Adult *Aphthona nigriscutis* beetles [2-5] are a yellowish-brown or bronze color with a small black "spot" on the dorsal surface, just in front of the hard wing covers. The underside is very dark (nearly black), and the legs are brown. Adults of *Aphthona nigriscutis* and *A. cyparissiae* are very similar in coloration and are difficult to separate without detailed microscopic examination.

Larvae: Though adult flea beetles may defoliate leafy spurge plants, this damage has little or no impact on spurge survival. Larval root-feeding, however, does kill leafy spurge plants. Larval feeding causes death **directly**, by disrupting the plant's ability to acquire and transport water and nutrients, and **indirectly**, by providing entry points for soil-inhabiting fungal pathogens. The stems of leafy spurge plants killed by *Aphthona* larvae exhibit a characteristic appearance [2-6]. Dead stems remain erect throughout the winter and into the following summer, while stems of healthy plants age in the fall and are knocked down by winter snowfall.

All *Aphthona* species spend most of the year in the larval stage, on or near leafy spurge roots. *Aphthona* larvae are so similar in appearance that the individual species cannot be distinguished. Larva are 5 mm or less in length, with creamy-white colored bodies, yellowish heads, and a legless appearance. *Aphthona* larvae may appear in the soil near spurge roots [2-7], or may burrow in larger roots and root buds [2-8]. Carefully digging up and examining leafy spurge roots and surrounding soil may reveal the larvae. However, because of their small size and underground feeding behavior, *Aphthona* larvae often escape detection unless populations are high.

***Oberea erythrocephala* (Root-Boring Beetle)**

Life Cycle:

Oberea erythrocephala exhibits complete metamorphosis. It may complete one generation per year in Europe, but appears to require 2 years to complete its life cycle in the United States. Adults are present during early to mid-summer, when they feed on leafy spurge leaves and flowers. They are active fliers and move readily among leafy spurge plants, so adult feeding is never conspicuous. After mating, the female chews one or more times partly or completely around the upper part of a leafy spurge stem. She then chews a hole into the stem just above these "girdles" (about two-thirds up the height of the stem), into which she deposits a single egg. The female often chews on the stem above this hole after laying the egg. The female generally lays only a single egg on each leafy spurge stem, but each female may lay up to 40 eggs. White "latex" oozes from the area of chewed stem tissue and the egg-laying hole, and the stem above the "girdles" typically wilts and curls. Both symptoms are characteristic of *Oberea* egg-laying activity.

Eggs hatch in about 2 weeks, and young larvae tunnel downward inside the leafy spurge stem until they reach the root crown area (just below the soil surface). *O. erythrocephala* larvae then complete their development within the root crown and inside the largest lateral roots, creating larval "mines" that consume most of these tissues. Larvae become dormant during the winter, and resume feeding and development in the spring. If the larvae require 2 years to complete their development, they continue feeding throughout the second summer, enter dormancy over a second winter, and complete development the following spring.

In late spring, larvae construct a pupal "cell" in the upper part of the root crown and molt to the pupal stage. Pupation lasts for about a month; newly-hatched adults chew through the remaining root crown tissue and emerge from the soil.

Identification:

Adults: *Oberea erythrocephala* adults are slender beetles (10-12 mm long) with long, dark antennae [2-9]. They are slate-gray above with a reddish-orange head and thorax; the underside is lighter, and legs are yellow. They usually appear on the upper part of spurge stems, feeding on foliage or flowers, or they may fly just above spurge plants. Adult feeding has no impact on leafy spurge plants. Egg-laying damage by females usually kills the upper part of a spurge stem, which may reduce flower and seed production but has little or no impact on plant survival.

Larvae: *Oberea erythrocephala* larvae [2-10] are white with a yellow head and obvious segmentation, and are up to 20 mm long. They burrow inside the leafy spurge root crown and larger roots.

Larval feeding destroys much of the tissue in the root crown, disrupting water and nutrient movement in the plant. This damage may be sufficient to kill smaller spurge plants. Larger plants may not die immediately, but will produce fewer and smaller shoots. This loss of photosynthetic capacity, coupled with future *Oberea* attacks, may eventually deplete a spurge plant's nutrient reserves and kill the plant.

***Spurgia esulae* (Bud Gall Midge)**

Life Cycle:

Spurgia esulae also exhibits complete metamorphosis, having distinct egg, larval, pupal, and adult life stages. It has three generations per year in Montana, and may have additional generations in warmer areas. Adults first appear in late spring or early summer, and again at irregular intervals through late summer. Adults usually live only a day or two under field conditions. After mating, females lay groups of orange eggs on leafy spurge stems and leaves, near an apical bud. An individual female may lay 20-100 eggs, usually in groups of 20 or more.

Eggs hatch in less than a week, and young larvae crawl to the adjacent apical bud and enter the dividing tissues. Larval feeding in the bud induces the formation of a "gall," which is a proliferation of highly-modified, leaf-like tissues. Larvae feed on various plant tissues inside the gall. Generally, larvae complete their development in about 3 weeks, though galls may persist longer. In earlier generations, larvae spin silken cocoons within the gall, in which they pupate. The pupal stage lasts about a week, after which newly-hatched adults exit the gall. In the final generation, larvae leave the gall before completing development and enter the soil. These soil-inhabiting larvae survive the winter in a dormant state and resume development the following spring. Pupation takes place in the soil, from which newly-hatched adults emerge to initiate the first *S. esulae* generation.

Identification:

Adults: *Spurgia esulae* adults [2-11] are very small (1-2 mm), mosquito-like flies that are a dark gray color with a reddish abdomen. They are short-lived and rarely found in the field.

Galls: *Spurgia esulae* induces a leafy spurge plant to product a characteristic "gall" [2-12] on stem tips or, especially later in the summer, on the tips of lateral branches. Galls appear from late spring throughout the summer. The galls have a shape somewhat similar to a small closed pine cone or artichoke, with overlapping, warty, modified leaves. *S. esulae* galls are usually a lighter green color than "normal" leafy spurge foliage, and may acquire a "bleached" appearance over time. Gall size is quite variable, ranging from about 6 mm (0.25 in.) to nearly 40 mm (1.5 in.) long; first-generation galls are typically larger than those produced by subsequent generations.

Bright orange, legless larvae (1-2 mm long) and light orange pupae, contained within white cocoons, develop inside the galls. They cannot be seen unless the gall "leaves" are peeled back. The number of larvae contained within a gall is highly variable, but typically averages from 10-30 insects. After larvae and pupae complete development, galls become yellow, then progressively browner, as they dry out and fall apart.

Spurgia esulae galls do not kill leafy spurge plants, but they may "stress" attacked plants so that the plants become somewhat more susceptible to other biocontrol agents. In addition, galled stems usually do not product flowers, so seed production may be reduced when gall densities are high.

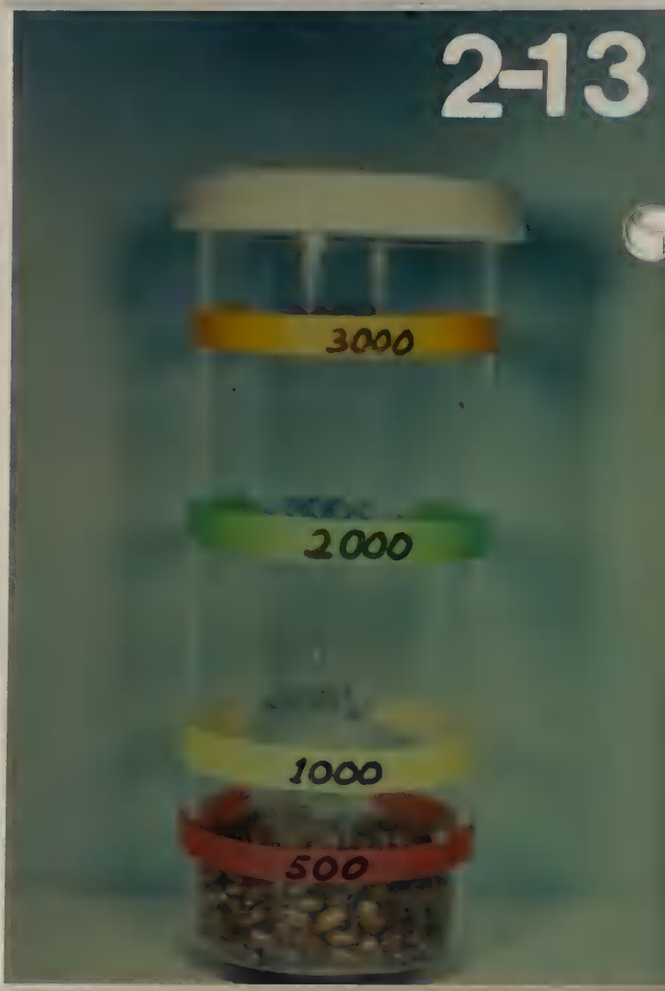

2-1


2-3


2-2


2-4


2-5



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APPENDIX 3: SUMMARY OF LEAFY SPURGE BIOCONTROL AGENTS (1993-1994)

Introduction

Use this appendix as a quick reference to the biocontrol agents currently in use for control of leafy spurge. The table includes a pronunciation guide for the scientific names, as well as a summary of each insect's classification. You will also find information on the range, release history, and life cycle of each biocontrol agent. APHIS has used other agents in the past and plans to use additional agents in the future, but you will be working with one or more of the insects listed in this appendix when you establish field insectary sites.

Species:	Pronunciation:	Order: family:	Native range:	1st yr rel. U.S.:	# Gen./ yr.:	Overwintering stage:
Aphthona cyparissiae (Koch) (flea beetle)	Aff-thone-ah sy-pah-**riss**-ee-ee	Coleoptera: Chrysomelidae	Most of Europe	1986	1	Larva, in soil
Aphthona czwalinae Weise (flea beetle)	Aff-thone-ah che-vah-**leen**-ah	Coleoptera: Chrysomelidae	Cent. Europe to cent. Asia	1987	1	Larva, in soil
Aphthona flava Guillebeau (flea beetle)	Aff-thone-ah **flay**-vah or **flaw**-vah	Coleoptera: Chrysomelidae	Most of Europe	1986	1	Larva, in soil
Aphthona lacertosa (Rosh.) (flea beetle)	Aff-thone-ah lass-er-toe-sah	Coleoptera: Chrysomelidae	Cent. and s. Europe	1993*	1	Larva, in soil
Aphthona nigriscutis Foudras (flea beetle)	Aff-thone-ah ny-gri-**skew**-tiss	Coleoptera: Chrysomelidae	Most of Europe	1989	1	Larva, in soil
Oberea erythrocephala (Schrank) (root-boring beetle)	Oh-bah-ree-ah er-rith-row-**seff**-a-la	Coleoptera: Cerambycidae	Cent. Europe to cent. Asia	1980	<1	Larva, in roots
Spurgia esulae Gagne (bud gall midge)	Spur-gee-ah **ess**-yoo-la	Diptera: Cecidomyiidae	Cent. and e. Europe	1985	3+	Larva, in soil

*Accidentally released with earlier *A. czwalinae* releases.

APPENDIX 4: OPERATION OF TRANSPAK II GLOBAL POSITIONING SYSTEM (GPS) UNITS

Introduction

To determine the locations of Field Insectary Sites (FIS), use the Transpak II GPS Unit. Detailed instructions for the operation of Transpaks are provided by the manufacturer. **Please review these instructions before operating the unit.** All Transpak users must operate the units in the same manner to provide uniformity in the data. Be sure that the unit you are using is configured in the following way:

SETUP
LAND / AUTO
MG / ENGLISH /DMD
LOC = UTC +/- # / ASCII
NAD - 27 CONUS / OOS

Before visiting a site to be determined through global positioning, note the site's elevation from a topographical map. Three dimensional fixes are best, but it is sometimes necessary to enter the elevation manually if too few satellites are available to get a 3D fix. Error in latitude/ longitude readings will occur in 2D mode if the elevation has changed significantly since the last 3D fix. **The accuracy of the 2D solution depends on the accuracy of the estimated elevation!** Review section **POS** (pages 16-19) in the operation and maintenance guide for more information on manually entering elevations and Auto/2D/3D operation of Transpak II GPS units. Obtain maps from your records to assist you in locating the FIS in preparation for site visits. The local APHIS office should have copies of FIS maps and the coordinates (Township, Range, and Section #) for each FIS.

Saving GPS Determined Locations in Memory

The Transpak II GPS unit is able to store 999 waypoints in memory. To save the current location, switch the mode indicator to **WPT**, toggle the L/R switch until the flashing cursor highlights **FIX** and flip the +/- switch. The current location will be saved as the next available waypoint. Record the waypoint number and site name in your field book or on the enclosed form and/or use the **EDIT** function to assign a label (site name) to the waypoint for identification. **The Transpak II unit will not store elevation in memory. Read the elevation from the POS screen and record in your field notes along with waypoint number and site name.** Review section **WPT** (pages 30-37) for more information regarding waypoint functions.

Reporting Waypoint Locations and Elevations

Use the enclosed form for reporting waypoint latitude-longitudes and elevations. Include the township, range, and section coordinates for all releases made before the 1992 field season. The township, range, and section coordinates are the current reference in the database for the site location and must be included in the report. New release locations (1992 and later) will not require township, range, and section data--only latitude-longitude derived by Transpak GPS.

Locating GPS Derived Biological Release Locations

One of the features of the Transpak II GPS unit is its ability to navigate or steer the operator from the current position to a desired location. The desired location must be in the waypoint library for the unit to navigate to that point. To activate this feature, flip the mode switch to the **NAV** position. To navigate to a known point, key in the waypoint number at the **TO** blank on the **NAV** screen. The velocity, heading, range, and bearing are displayed on the **NAV** screen. Although this function is designed for naval or aerial use, you can use it to locate positions on foot. It does take some practice, however, and you should attempt the procedure prior to actual field application. The following definitions are useful in the application of this function:

RANGE -- The distance from the present position to the desired location.

BEARING -- The direction to the desired location relative to magnetic north expressed as a compass reading in degrees.

HEADING -- The horizontal direction a moving ship, plane, or person is pointed, also expressed as a compass reading in degrees.

If the desired location is not in the waypoint library, you can key it in through the **EDIT** option of **WPT** mode (see pages 30-37 for more information). A hand-held compass may be useful in helping you locate biological release points. The **DIST** function is also useful in determining the distance and bearing from the present position to a release site location and is sometimes easier to use than the **NAV** function. Save the current position as a waypoint prior to using the **DIST** function. For more information on **NAV** and **DIST** functions, consult the Transpak II operation and maintenance guide (pages 20-25 and 28).

Please direct all questions or comments regarding the operation of Transpak II GPS units to the Bozeman Biological Control Facility.

APPENDIX 5: APHIS PROCESS TO RELEASE EXOTIC NATURAL ENEMIES OF WEEDS FOR ESTABLISHMENT AND REDISTRIBUTION

Introduction

This appendix summarizes the "Phase" concept of weed biological control. APHIS applies the "Phase" concept to the biological control of other weeds as well as leafy spurge. In Phase 1, initial releases of natural enemies are made into limited, protected, field insectaries. APHIS performs releases and management. Phase 2 involves cooperative establishment of field insectaries with materials from Phase 1 initial field insectaries. Technology is transferred from APHIS to cooperators, and management is by cooperators. In Phase 3, natural enemies are redistributed from Phase 2 field insectaries. Total management is by the cooperator.

Phase 1

The introduction and release of exotic natural enemies for weed control involve initial releases of only a few organisms, at well defined and protected field sites, that would be free of any pesticide applications and open grazing. These initial sites would be carefully selected by APHIS with the assistance of various cooperators. These sites are limited in number depending on the availability of specimens collected from foreign sources and cleared through importation procedures. These sites may be in several States or only one State, possibly confined to only one county. APHIS' responsibility in Phase 1 is to protect and maintain these critical sites for several years in order to promote natural population increases of a particular species. The insects produced at such a location then become available for Phase 2. The time required between initial introduction and population development sufficient for redistribution to Phase 2 insectaries may be 3-5 years dependent upon species involved, initial numbers of agents released, and factors affection population development.

Phase 2

The establishment of field insectary sites (FIS) from initial Phase 1 releases would occur in each state that is infested by the target weed of concern. The establishment of these Phase 2 FIS is a joint cooperative effort by APHIS and state departments of agriculture and/or research cooperators. These sites will serve two purposes. Each site will be the source of additional natural enemies in 3 to 5 years for continued redistribution in Phase 3. These sites may also serve as demonstration plots, showing the potential impact of the natural enemy on the targeted weed in that particular State. During Phase 2, APHIS will serve as a source of information and techniques in support of State departments of agriculture and cooperators.

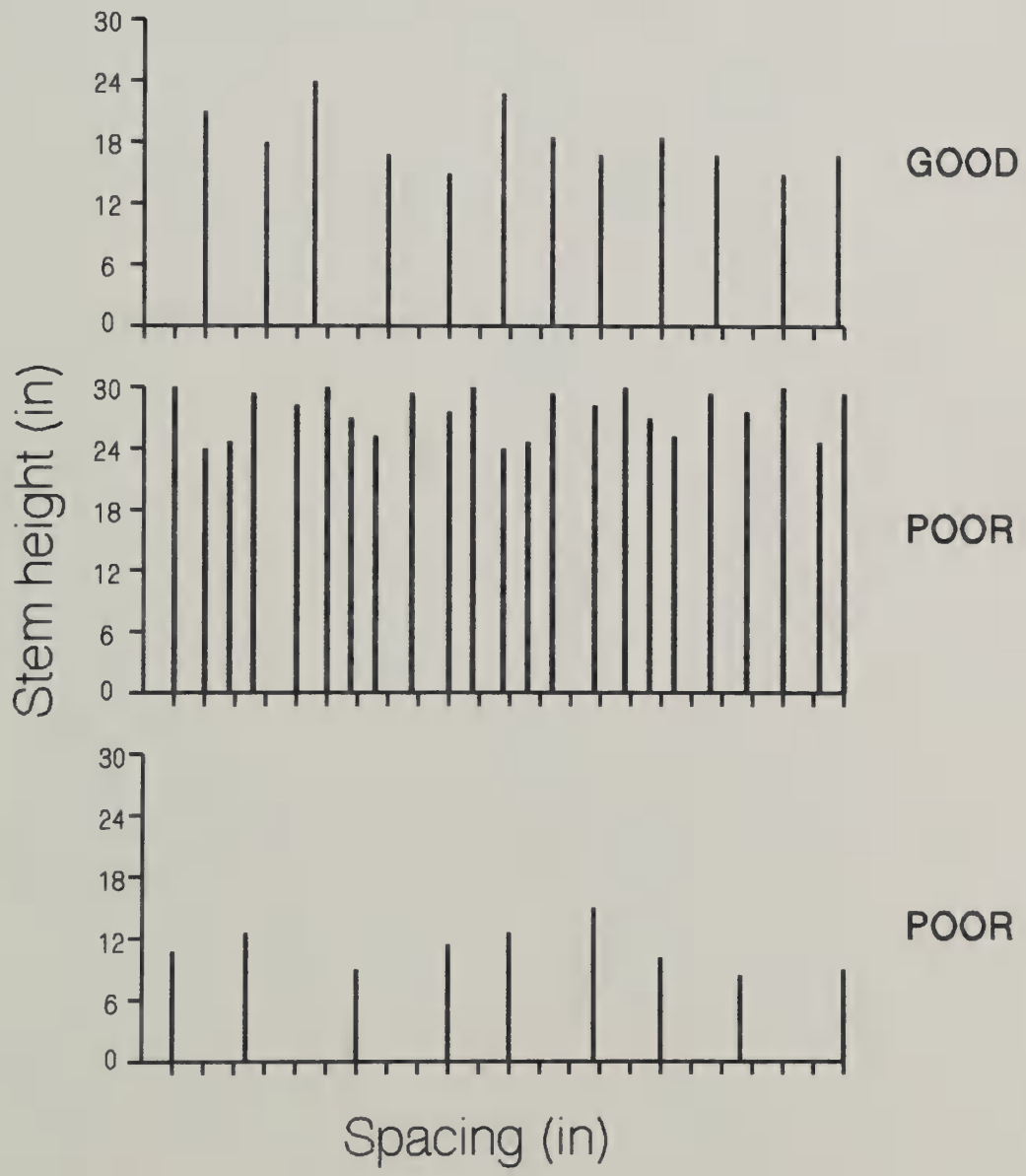
Phase 3

Natural enemies collected from Phase 2 FIS will be redistributed within each State. These releases may be directed at the county level for establishment of each species. Establishment of additional FIS within each county and/or at individual grower sites will be determined by the State department of agriculture and/or research cooperator. The collection and redistribution of natural enemies from the FIS developed in Phase 2 is the sole responsibility of the State departments of agriculture and/or research cooperators. At this time, commercial insectary operations and the general public could approach either responsible party for starter culture material and/or assisting in the redistribution efforts within that State.

APPENDIX 6: IDEAL STEM SPACING AND HEIGHT

Introduction

This appendix is a graphical representation of good and poor leafy spurge stem spacing and height. Use this appendix to help choose a "moderately dense" stand of leafy spurge when you are selecting a suitable site for the insectary (see pages 2.3 and 3.3). Ideally, individual stems should be 1-2 inches apart; never more than 6 inches apart. The best plant height is 15-24 inches.



APPENDIX 7: EXAMPLES OF FORMS

Introduction

This appendix provides you with examples of the following forms used for the LS Project:

- USDA-APHIS BIOCONTROL OF WEEDS: FIELD INSECTARY SITE PRELIMINARY INFORMATION SHEET (FISPIS)
- BIOLOGICAL SHIPMENT RECORD--NON-QUARANTINE (Form AD-943)
- SUPPLEMENTAL DATA (Form AD-943A)
- Leafy Spurge Biocontrol Agent RECOVERY AND SAMPLING REPORT

Refer to the appropriate sections of the manual for specific instructions on completing these forms.

Examples **Field Insectary Site Preliminary Information Sheet (FISPIS):**

USDA-APHIS BIOCONTROL OF WEEDS: FIELD INSECTARY SITE PRELIMINARY INFORMATION SHEET (FISPIS)

Target Weed: X **Leafy spurge** _____ **Diffuse/spotted knapweed** (Release Code: 30031-63)

Contact person:
Name: Joe Schmoe, Weed Supervisor
Address: Gallatin Co. Fairgrounds
City: Bozeman
State: MT Zip: 55555
Phone: 406-555-4321

Legal landowner, if different (person or agency):
Name: Ted Turner
Address: P.O. Box 199
City: Gallatin Gateway
State: MT Zip: 55555
Phone: 406-555-1234

SITE LOCATION:
Site name: Sheep Draw
State: MT County: Gallatin Twp.: T02S Range: R06E Sect.: 05 Qtr-sect.: SW
Latitude: 45°41' 22.9" N Longitude: 110°59' 39.9" W (derived from GPS?: X Yes ___ No)

▸ **Please draw ■ sketch map of site on back of sheet, or attach a map, that includes road access to site** ◂

SITE CHARACTERISTICS **(please check answer or supply requested information; if you are unable to answer a question, please leave it blank.)**

A. PHYSICAL

1. Soil texture: ___ **Fine** (clay) X **Medium** (silt/loam) ___ **Coarse** (sand/gravel)
2. Typical soil moisture regime: X **Well-drained** ___ **Moderately well-drained** ___ **Poorly drained**
3. Risk of spring flooding: X **None** ___ **Low-moderate** (occasional years) ___ **High**
4. General topography: X **Hilly** ___ **River valley** ___ **Level** ___ **Islands**
5. Specific site topography: ___ **Slight slope** X **Steep slope** ___ **Level**
   (Is release site situated on a: ___ **hilltop** or in a ___ **valley**?)
6. Is the slope facing: ___ **N** X **S** ___ **E** X **W** (check two for combinations, e.g. SW)
7. Estimated bare ground (exposed soil) at site: 5 % of surface
8. Altitude (if known): 5745 ft (derived from GPS: X Yes ___ No)
9. Annual precipitation (if known): ___ **<10"** ___ **10-15"** ___ **15-20"** X **>20"**

B. BIOLOGICAL

10. Is weed infestation: X **Continuous** ___ **Interrupted** (weed patches separated by open or non-weed areas)
11. Size of weed infestation: ___ **1 ac or less** ___ **2-5 ac** X **5 ac or more**
12. Weed density: ___ **0-40** stems/ft² X **40-100** stems/ft² ___ **100 or more** stems/ft²
13. Vegetation cover (ground area shaded by plants): ___ **0-25%** ___ **25-75%** X **75-100%**
    (Estimated vegetative cover attributable to weed: 90 %)
14. Typical weed height: X **<2 ft.** ___ **2 ft or greater** (Average weed ht.: 22 in, measured on (date): 6/24/93
15. Is weed flowering?: X **Yes** ___ **No**
16. Trees or woody shrubs, in release site: ___ **Yes** X **No**; in surrounding areas: X **Yes** ___ **No**
    (If present, are trees: X **Conifers** ___ **Deciduous**)
17. Shade from trees and shrubs in release site: ___ **Full** ___ **Partial** X **None**
18. What is the "natural" vegetation in the area, based on the most abundant and conspicuous (dominant) plants:
    ___ **Forest** ___ **Savanna** (trees and grassland) ___ **Shrub** X **Shrub-grassland** ___ **Grassland/prairie**

C. CULTURAL

20. Current land use: X **Pasture** ___ **Recreational** ___ **Roadside/right-of-way** ___ **Idle cropland** ___ **Other**
    (If **Other**, please describe: _____)
21. Herbicides applied within the last 2 (two) years?: X **Yes** ___ **No**
    (If **Yes**, list **herbicide**: Tordon 22K **Month/year applied**: 7/92)
22. Other treatments within last 12 (twelve) months?:
    a. Mowing: ___ **Yes** X **No** c. Insecticides (e.g. grasshopper control): ___ **Yes** X **No**
    b. Grazing: X **Yes** ___ **No** d. Timber harvesting: ___ **Yes** X **No**
    (If **Yes**, what grazers: X **Cattle** ___ **Sheep or goats** ___ **Horses**)

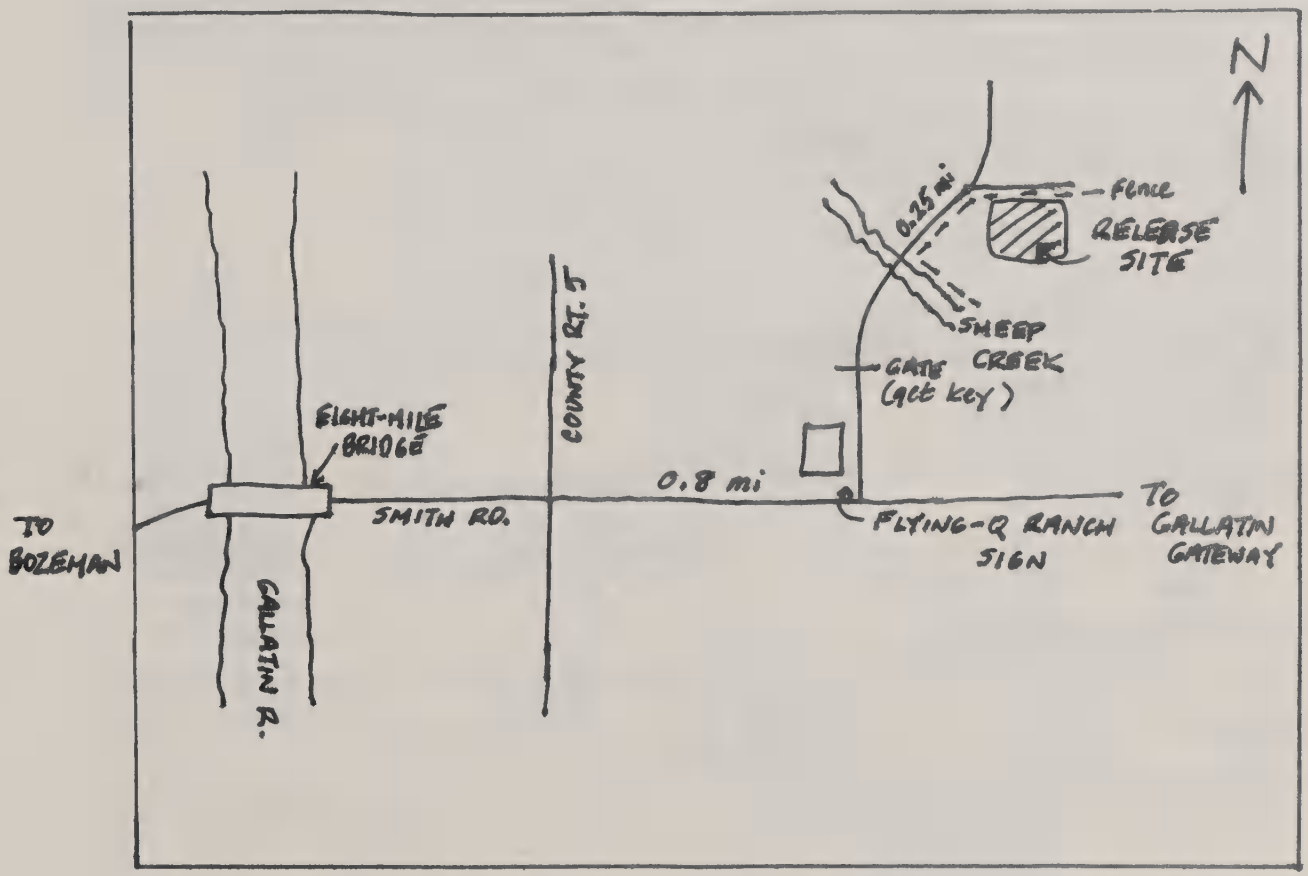
D. OTHER

23. Accessible by vehicle within 1/4 mile (including 4WD)?: X **Yes** ___ **No**
24. Unauthorized access "controlled" (fences, etc.)?: X **Yes** ___ **No**
25. This release of biological control agents requires a ■ **(five) year commitment**, that includes:
    a. No grazing from June through September, unless release site is fenced
    b. No herbicide or insecticide applications **c**. Restricting access by unauthorized collectors
    d. Allowing periodic access by APHIS and cooperating personnel
    Is the cooperator aware of, and willing to make this commitment?:
    X **Yes** ___ **No** X **Yes**, but with these **restrictions**: Gov't vehicles only; get key from ranch manager

Signed: Edward R. Johnson Date: 6/24/93

Rev. 03/02/93

Field Insectary Site Preliminary Information Sheet (FISPIS) (back):



Form AD-943 (Biological Shipment Record - Non-Quarantine:

OMB No. 0518-0013 (EXP. 4/30/93)

U.S. Department of Agriculture

BIOLOGICAL SHIPMENT RECORD – NON-QUARANTINE

SECTION I – REPORT OF MATERIAL RELEASED OR SHIPPED

1. FROM (Name & address of Shipper/Releaser)
Richard Hansen
USDA, APHIS, PPQ
Forestry Sciences Laboratory
Montana State University
Bozeman, MT 59717-0278

2. BENEFICIAL - A. Gen., sp., subsp., auth.: Aphthona cyparissiae (Koch)
B. Order: Family: Coleoptera: Chrysomelidae
C. Determined by (Name and affiliation if known): R. White, USNM

3. SHIPPER/RELEASER FILE NO. (see instructions): BBCFAPCY93-06

4. TYPE OF BENEFICIAL
☐ PArasite ☒ WEed feeder
☐ PRedator ☐ POllinator
☐ Microbial ☐ OTher
(Explain MI or OT):

Part A. From U.S. Field Collection (Collected for field to field recolonization or laboratory culture) And/Or Part B. From Laboratory Culture

5. COLLECTION LOCALITY(S) - State, County, nearest Town (If more than 2 collection sites, give State & County only)
MT, Sweet Grass Co., Big Timber

9. SOURCE FILE NOS.
☐ AD-942, AD-943: Nos.
☐ Part A ☐ Other:

10. COUNTRIES/REGION/STATE OF ORIGIN
Hungary

6. DATES OF COLLECTION (m,d,y): 07/15/93

7. COLLECTORS (Names and affiliations): R. HANSEN, APHIS-PPQ

11. ORIGINAL COLLECTORS (Names and affiliations): E. Shrdlu, IIBC

12. NO. LAB GENERATIONS (At shipper/releaser location)
☐ $F_1 - F_{10}$ ☐ $F_{51}+$
☐ $F_{11} - F_{50}$

8. U.S. FIELD HOSTS/PREY AT COLLECTION
A. Genus, species: Euphorbia esula L.
B. Stage/part attacked (see codes): RT/FO

13. LABORATORY HOST/PREY
A. Genus, species
B. Stage/part attacked (see codes)

SECTION II – REPORT OF SHIPMENT

14. SHIPPED TO (Name & address)
John Doe
Gallatin Co. Courthouse
Bozeman MT 55555
VIA: Fed Ex Overnight

15. NO. & STAGES SHIPPED (use codes on reverse): Est 1000 A

16. DATE SHIPPED (m,d,y): 07/16/93

17. SHIPPER'S REMARKS: Coll'd at 0900 hrs; heavy dew

18. SPECIMENS RETAINED BY SHIPPER
☒ No
☐ Yes _____ nos.

19. DATE RECEIVED (m,d,y): 07/17/93

20. NO. & STAGES (use codes)
A. Rec'd. Alive: Est 990 A
B. Emerged (Beneficials): Est

21. RECEIVER'S REMARKS: Good condition, >90% survival

22. SPECIMENS RETAINED BY RECEIVER
☐ No
☒ Yes 25 A nos.

23. INTENDED USE
A. ☒ Immediate release (complete Sect. III)
Lab culture/study (complete Blk. 24)
B. ☐ Release intended
C. ☐ No release intended

24. INTENDED LAB HOST/PREY - Gen., sp.: N/A

SECTION III – REPORT OF RELEASE/RECOLONIZATION (See instructions on cover sheet; use Form AD-943A for more details)

	SITE 1	SITE 2	SITE 3
25. Types of release	☒ Field ☐ Greenhouse ☐ Cage ☐ Other:	☐ Field ☐ Greenhouse ☐ Cage ☐ Other:	☐ Field ☐ Greenhouse ☐ Cage ☐ Other:
26. Locations (State, County, nearest Town or physical feature, map coordinates) (Use AD-943A for more details; see instructions on cover sheet)	MT, Gallatin		
27. Number & stages released (Use codes; see instructions for recording multiple releases.)	Est 965 A	Est	Est
28. Dates of releases (m,d,y) (See instructions for recording multiple releases.)	07/17/93		
29. Target hosts/prey at release A. Primary - Genus, species	Euphorbia esula		
B. Other - Genus, species			
C. Families			
30. Food (plant/animal/other) of target host/prey at release	N/A		
31. Released by (Name and affiliation)	John Doe, Gallatin Co. Weed Supervisor		

32. REMARKS (Use AD-943A for more details)
Released with A.B. Normal, USDA-APHIS; location marked with green pipe

33. REPORTED BY
A. Name A.B. Normal
B. Date (m,d,y) 07/17/93

Form AD-943 (10/83) Part 2 - SHIPPER/RELEASER COPY

Form AD-943A (Supplemental Data):

OMB No. 0518-0013 (EXP. 4/30/90)

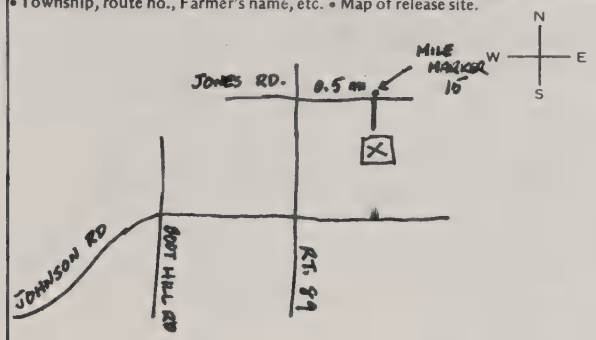
SUPPLEMENTAL DATA

NOTE: • Do not fold this sheet over form when writing-carbons will distort entries.
• If additional copies are needed, photocopy and staple to form.

Shipper's File Number (From AD-943): BBCFAPCY 93-06

Section A – RELEASE SITE DETAILS, SITE NO. _____.

• Township, route no., Farmer's name, etc. • Map of release site.



WEATHER: Partly cloudy
TEMP. 75°F
WIND Light NW
SKY Broken Clouds
TIME OF RELEASE: 1400 MST
CONDITION OF CROP FIELD: Dry
CONDITION OF RELEASE MATERIAL: Good
PREDOMINANT TARGET HOST/PREY STAGE PRESENT:
TARGET HOST/PREY ABUNDANCE:
ADDITIONAL HOST/PREY PRESENT:

OTHER COMMENTS:
REPORTED BY & DATE:

Section B – DETAILS OF ADDITIONAL RELEASES *(Attach additional sheets as needed.)*

	SITE 4	SITE 5	SITE 6
Types of release	☐ Field ☐ Greenhouse ☐ Cage ☐ Other:	☐ Field ☐ Greenhouse ☐ Cage ☐ Other:	☐ Field ☐ Greenhouse ☐ Cage ☐ Other:
Locations (State, County, nearest Town or physical feature, map coordinates)			
Number and stages released *(See codes)*	[Est]	[Est]	[Est]
Dates of release *(m,d,y)*			
Target hosts/prey at release			
A. Primary – *Genus, species*			
B. Other – *Genus, species*			
C. Families			
Food (plant/animal/other) of target host/prey at release			
Released by			

Section C – DETAILS OF MULTIPLE RELEASES *(Attach additional sheets as needed.)*

(Alternatives 1 and 2)

SITE__ Dates of release	SITE__ Nos. Released *(stages)*	SITE__ Dates of release	SITE__ Nos. Released *(stages)*	SITE__ Dates of release	SITE__ Nos. Released *(stages)*

(Alternative 3)

Counties	Locations	Dates of Release	No. Released *(Stages)*

REMARKS:
REPORTED BY
A. Name
B. Date *(m,d,y)*

Form AD-943A (10/83)

Leafy Spurge Biocontrol Agent Recovery and Sampling Report - 1993:

USDA-APHIS-PPQ
Bozeman Biocontrol Facility
Forestry Sciences Lab - MSU
Bozeman, MT 59717

Leafy Spurge Biocontrol Agent RECOVERY AND SAMPLING REPORT - 1993

Release Site Location: Release code: 30031-63
State: MT County: GALLATIN
Site name: SHEEP DRAW
USGS coordinates: T 025 R 06E Sect. 05 Qtr-sect. SW
Latitude: 45° 41' 22.9"N Longitude: 110° 59' 39.9"W (GPS derived? X YES ___ NO)

INSECT(S) RELEASED:

___ Flea beetle *Aphthona nigriscutis*
___ Flea beetle *Aphthona flava*
X Flea beetle *Aphthona cyparissiae*
___ Flea beetle *Aphthona czwalinae*
___ Long-horned beetle *Oberea erythrocephala*
___ Gall midge *Spurgia esulae*

Date of original release: 1991
___ CAGED or X OPEN release

SAMPLING INFORMATION
[Sampling procedures are described below]

SAMPLING DATE: 07/15/94 SAMPLING TIME (approx.): 1300 MDT

Weather conditions: X Clear ___ Partly cloudy ___ Overcast ___ Rain ___ Other (specify:)
Air temperature (°F): ___ <60° ___ 60-70° X 70-80° ___ 80-90° ___ >90° (Actual temp., if measured: 73°F
Wind: ___ Calm X Light ___ Moderate ___ Strong

Visual observation of insect, before sweeping? X YES ___NO

Number of:
Net sweeps: 80
TOTAL number of insects swept (sum from reverse): 93
For *Spurgia esulae*, number of galls observed:
___ None ___ <25 galls ___ 25-100 galls ___ >100 galls

Observer: Rich Hansen
Affiliation: USDA-APHIS-PPQ
Phone: 406-994-5037

GENERAL SAMPLING INSTRUCTIONS

First, look over the release area to see if biocontrol insects are visually apparent. Next, five sampling points will be swept along four lines in N, S, E, and W direction from release point (20 points total). For each line, begin as close to the release point as possible. Using a 15-in diameter sweep net, make **four** sweeps in front of you (back and forth twice). Each net sweep should proceed in a downward arc, so that the net moves vigorously through the vegetation as close to the ground as possible. Carefully examine the net and count the biocontrol insects present, then empty the net to release counted insects. Move 5 to 6 ft out and repeat above steps. Continue until five points have been sampled, then repeat over the remaining cardinal directions. A diagram of the sampling procedure and a chart on which to record insect counts is provided **on the back of this form.**

During the sweeping process, collect a **maximum** of 15 beetles from the site and place them in 70% alcohol in a glass vial. (NOTE: **If sampling an *Oberea erythrocephala* release, collect only one or two adult beetles.**) Label the vial with the **release code, site name, and date,** and send specimens to the Bozeman Biocontrol Facility along with this form. If no specimens are collected, return only the completed form.

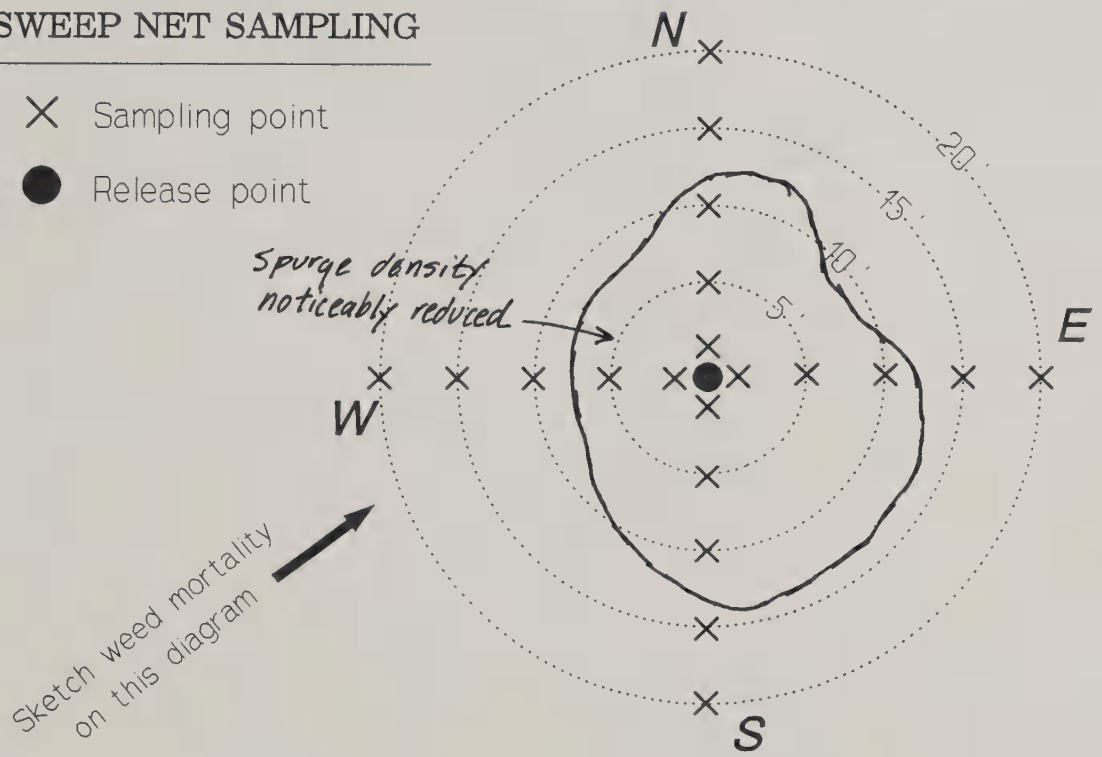
Using the diagram on the back of this sheet, sketch out any weed mortality that is visible around the release point.

NOTE: At *Spurgia esulae* releases, do **not** use the sweep-net sampling procedure described above. Instead, survey an area roughly 50-75 ft in diameter and centered at the original release point and note the relative abundance of galls on spurge stems. Up to five galls may be collected, placed in an alcohol-filled glass vial, and returned to BBCF to confirm identification.

Rev. 03/02/93

Leafy Spurge Biocontrol Agent Recovery and Sampling Report - 1993 (back):

SWEEP NET SAMPLING



Insect counts

Direction:

Distance from release point

	N	S	E	W	
0	1	5	2	1	9
5	6	11	4	3	24
10	6	15	19	9	49
15	2	7	0	1	10
20	0	1	0	0	1
	15	39	25	14	(93)

APPENDIX 8: HOST PLANT SPECIFICITY TESTING

Introduction

APHIS incorporates rigorous "safety testing" procedures into the process of classical biological control. You should feel free to assure any landowners or cooperators with whom you interact that the leafy spurge biocontrol agents described in this manual will not attack any economically important plants or native plants outside of the genus *Euphorbia*.

TAG Evaluation

Any foreign biocontrol agent being considered for importation and release against leafy spurge must first be evaluated by a multi-agency Technical Advisory Group (TAG). The TAG considers whether the organism in question is a potential threat to U.S. crop, ornamental, and native plants, and then recommends whether or not the agent should be introduced. USDA-APHIS, acting on a positive decision by TAG, then issues permits for the importation and release of the biocontrol agent.

Most of the evidence considered by TAG consists of "screening" or host-specificity experiments with the biocontrol agent in question. The International Institute of Biological Control (IIBC) in Switzerland or the USDA-ARS laboratory in Montpelier, France, tests for host-specificity of biocontrol insects. For some insects, USDA-ARS or university laboratories in this country may conduct additional tests under quarantine conditions. Entomologists test a variety of plant species, including leafy spurge and related species, plants reportedly eaten by insects related to the species being examined, and a range of crop and ornamental plants. The entomologists try to include a number of native species related to leafy spurge. Tests assess the "ability" of plant species to support: (1) adult insect feeding, if relevant; (2) egg deposition by the adult female insect; and (3) survival and development of the larval insect (i.e. completion of the insect's life cycle). The last factor is probably the most important in determining the likelihood of a biocontrol agent feeding on a plant species under field conditions.

Taxonomy of *Euphorbia*

Botanists have not fully resolved the taxonomy of the genus *Euphorbia*, but there are more than 100 native *Euphorbia* species in the United States and at least 13 that have been introduced. There are at least six subgenera within the genus *Euphorbia*, four of which have representatives in North America. The weed in question, leafy spurge (*Euphorbia esula*), is a Eurasian native that belongs to the subgenus *Esula*; there are at least 20 native *Euphorbia* in this subgenus. The annual *Euphorbia spathulata (=E. spatulata)* and the perennial *E. robusta* belong to the subgenus *Esula*, while *Euphorbia glyptosperma, E. missurica, E. serpens,* and *E. serpylliflora* are all annual plants belonging to the subgenus *Chamaesyce*.

Aphthona cyparissiae

Aphthona cyparissiae was approved for U.S. release in 1986. European screening tests concluded that this species of flea beetle feeds exclusively on hosts in the subgenus *Esula*. Entomologists tested five species of North American *Euphorbia*, though none was in the subgenus *Esula*.

Aphthona czwalinae

Aphthona czwalinae was approved for initial release in the United States in 1987. Screening tests in Europe apparently confirmed that this species feeds only on hosts in the subgenus *Esula*. No feeding was reported on four North American spurge species tested, though none was in the subgenus *Esula*.

Aphthona flava

Aphthona flava was also approved for U.S. release in 1986. Host-specificity tests show that this insect feeds only on spurges in the subgenus *Esula*. Of the U.S. subgenus *Esula* spurges tested, *A. flava* was able to complete its life cycle on four, including *Euphorbia robusta* and *E. spatulata*. Under field conditions, however, *E. spatulata* would probably not be a suitable host, since *A. flava* requires the year-round availability of host roots. Interestingly, *A. flava* did not complete development on *Euphorbia purpurea* and *E. telephiodes*, two native species in the subgenus *Esula* under review for protected status. Thus, *A. flava* has a host range that is restricted beyond the sub-generic level.

Aphthona lacertosa

Aphthona lacertosa received approval for U.S. release in 1993. This beetle is also restricted to hosts in the subgenus *Esula*. The five North American *Euphorbia* species tested were not suitable hosts, though none was in the subgenus *Esula*.

Aphthona nigriscutis

Aphthona nigriscutis was approved for U.S. release in 1989. European screening tests show that this species feeds only on *Euphorbia* species in the subgenus *Esula*. Of four tested North American species, none was in the subgenus *Esula*.

Oberea erythrocephala

Oberea erythrocephala, a root- and stem-boring beetle, received approval for U.S. release in 1979. European host-specificity tests included only three North American spurges (none in the subgenus *Esula*). Again, only *Euphorbia* species in the subgenus *Esula* appear to be suitable hosts. Since this insect probably requires 2 years to complete development in the northern United States, only perennial plants would be appropriate hosts.

Spurgia esulae

Spurgia esulae, the leafy spurge bud gall midge, was approved for U.S. release in 1985. Again, this insect selects host-plants restricted at least to the subgenus *Esula*. In U.S. testing, *S. esulae* completed its life cycle on four native subgenus *Esula* plants, including *Euphorbia robusta* and *E. spatulata*. The gall midge did not feed on the "threatened" *E. purpurea* and *E. telephiodes*.

Summary

Leafy spurge biocontrol agents appear to be restricted to host plants in the genus *Euphorbia*, subgenus *Esula*. Native spurges in the subgenus *Chamaesyce* (e.g. *E. glyptosperma, E. missurica, E. serpens,* and *E. serpyllifolia*) and other subgenera probably are not suitable hosts and are at little or no risk of attack. The Bozeman Biological Control Facility has received no reports of introduced leafy spurge biocontrol agents feeding on nontarget, native *Euphorbia* species. Certainly, you can be confident that approved leafy spurge biocontrol agents represent no threat to economic or native plants outside the genus *Euphorbia*.

INDEX

A - C

D - H

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Comment Sheet

Directions: Use this sheet to suggest an improvement or to identify a problem in the content of the manual. To mail, please follow the directions on the next page.

Description of problem (error, inconsistency, missing or insufficient information, etc.):

Description of improvement or recommended change (add attachments if necessary):

==

Reason for improvement or change:

AFTER COMPLETION, FOLD ON THE DOTTED LINES WITH THE ADDRESS SIDE OUTWARD. STAPLE OR TAPE TO CLOSE, AFFIX POSTAGE, AND DROP IN THE MAIL.

Professional Development Center
USDA-APHIS-R&D
7340 Executive Way, Suite A
Frederick, MD 21701

Attn: Bruce Attavian